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TURNIP MOSAIC VIRUSES[1]

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INTRODUCTION

The first transmissible virus diseases demonstrated on cruciferous plants were found in nature on turnip (*Brassica rapa* L.). They were reported in Indiana in 1921 by Gardner and Kendrick (*5*)[3] and from Washington, D. C., by Schultz (*14*). In the subsequent 20 years turnip mosaic was recorded from New York (*4*), Wisconsin (*8*), and Florida (*23*) in the United States, and from the Philippine Islands (*1*), Australia (*13*), New Zealand (*2, 3*), Japan, (*7, 15*), China (*12*), South Rhodesia (*9*), and Denmark (*6*). Since the work of Hoggan and Johnson (*8*), published in 1935, more attention has been given to the differentiation and characterization of viruses on cruciferous plants. As a result a number of distinct viruses or strains of viruses have been described on horseradish (*Armoracia rusticana* Gaertn., Mey., and Scherb.) (*8*), cauliflower (*Brassica oleracea* L. var. *botrytis* L.) (*16*), cabbage (*B. oleracea* L. var. *capitata* L.) (*10, 11, 20, 22*), Chinese cabbage (*B. pekinensis* (Lour.) Rupr.) (*21*), radish (*Raphanus sativus* L.) (*19*), annual stock (*Matthiola incana* (L.) R. Br. var. *annua* (L.) Voss (*18*), rape (*B. napus* L.) (*12*) and turnip (*8, 17*).

It is a significant fact that turnip was found to be host for each of these as they were described. In all cases distinct mottle or vein clearing or both occurred, and the plants were rather severely affected. On the other hand, each virus was distinguishable from the others on the basis of differences either in properties or host range or both.

In the course of studies on cabbage mosaic at Madison, Wis., reported recently (*22*), turnips showing signs of virus infection were commonly observed. Isolates from this host were made for comparison with those of cabbage mosaic. Isolates were also secured from the experimental plots at the Louisiana Agricultural Experiment Station. Of several collections four were studied critically. One of these conformed closely with cabbage virus A described recently (*22*), but the remaining three were distinct in certain respects from crucifer viruses previously described. The present paper reports details of the study of the four virus strains isolated from turnip.

METHODS AND MATERIALS

The methods of study of the viruses were similar to those recently detailed in another paper (*22*). The virus strains used in this investigation were as follows:

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[2] The writers express sincere appreciation to Eugene H. Herrling for making the photographs used in this paper.
[3] Italic numbers in parentheses refer to Literature Cited, p. 363.

Strain T1 was isolated from naturally infected turnip at the Louisiana Agricultural Experiment Station in March, 1939. At that time an isolation from cultivated mustard (probably *Brassica nigra* (L.) Koch) was also made but when found to be apparently similar to T1 it was discarded.

Strains T6, T8, and T9 were isolated from naturally infected turnip in experimental plots adjoining cabbage mosaic plots at the Wisconsin Agricultural Experiment Station in 1938 and 1939.

Stock cultures of isolates T6, T8, and T9 were kept on plants of *Nicotiana glutinosa* L. and cultures of T1 on turnip plants in insect-proof cages. Seedlings were usually inoculated in the third- or fourth-leaf stage and incubated at temperatures ranging from 24° to 28° C. Uninoculated controls were always carried for comparison with inoculated plants. Reisolations were attempted from all plants tested as possible hosts by inoculation to turnip or tobacco. The cabbage aphid (*Brevicoryne brassicae* (L.)) and the green peach aphid (*Myzus persicae* (Sulz.)) were used as insect vectors.

EXPERIMENTAL RESULTS

SYMPTOMS OF THE DISEASES

In the Field

Observations were made at Madison, Wis., in naturally infected plots from which T6, T8, and T9 were isolated. Since the symptoms later produced by these strains on turnip in the greenhouse were quite similar, it is assumed that this description of field symptoms will apply to the disease as produced by any one of the strains. After midsummer the disease was very severe. The affected areas in turnip plots were usually more or less circular in shape with severely stunted or dead plants at the center, indicating that infection had spread progressively outward from an initial center. Newly unfolding leaves of severely infected plants were dwarfed, malformed, and mottled while some leaves became entirely chlorotic, except for occasional, irregularly-shaped, raised islands of normal green or green darker than normal. Necrosis varied from small, more or less circular spots, to large, irregular areas and streaks, which finally involved whole leaves, causing them to dry up and abscise prematurely. When progressive dying of the older leaves continued, only a rosette of dwarfed, malformed leaves remained. Such leaves were not always severely mottled but were frequently darker green than normal throughout. The enlargement of the root and hypocotyl under these conditions was greatly suppressed. On the older leaves of less severely infected plants, vein clearing was sometimes evident, supplanted later by fine interveinal mottling. When White Milan and Purple Top White Globe varieties were grown side by side in the field at Madison, the former was much more severely affected (fig. 1).

When the mosaic disease was observed in December at Baton Rouge, La., in plots from which T1 was isolated, the symptoms were relatively mild as compared with those at Madison in midsummer. This may have been due in part to the cooler weather at Baton Rouge since it will later be shown that these mosaic isolates produced a severer type of disease above 20° C. than below. It was also undoubtedly due

FIGURE 1.—White Milan (left) and Purple Top White Globe (right) varieties of turnip naturally infected in plots at Madison, Wis., from which strains T6, T8, and T9 were isolated. Note that the greater susceptibility of White Milan to damage from mosaic has resulted in the death of most plants while the plants of Purple Top White Globe, though generally affected, are not seriously stunted

in part to the lack of virulence of T1 on turnip. Although a high percentage of the plants in the plot were infected, it was necessary to examine individual plants to detect the disease. Coarse mottled or irregular, frequently puckered, normal green or darker than normal green areas on a slightly chlorotic background occurred, but neither severe malformation nor necrosis was apparent. Little stunting could be detected.

IN THE GREENHOUSE

Young plants of the varieties White Milan and Purple Top White Globe were inoculated in the greenhouse with each isolate and kept at a constant temperature of 24° C. It was evident that White Milan and Purple Top White Globe were about equally affected by T1, but that White Milan was, as in the field, much more severely affected by T6, T8, and T9. T1 was much less severe on both varieties than T6, T8, and T9, as is shown for Purple Top White Globe in figure 2.

On plants inoculated with T1, vein clearing persisted on the first three or four leaves to develop symptoms, but as new leaves unfolded, it was soon replaced by a coarse mottle or by green bands along the veins (fig. 3). Necrotic spots were rare, but older leaves turned yellow and dried up prematurely. Aerial parts were only slightly dwarfed, but the root and hypocotyl were stunted considerably. The disease was never lethal.

The symptoms produced by T6, T8, and T9 were very much alike. Necrotic lesions on the inoculated leaf were noted only with isolate T9. These appeared in 5 to 8 days as small, circular, reddish-brown lesions, which later enlarged to a diameter of 2 to 3 mm. Only a

small percentage of inoculated plants gave rise to such symptoms, and in a number of inoculation series no lesions were observed. Usually they occurred only when old leaves were inoculated and then only at 24° and 28° C. Systemic invasion by either isolate was first apparent as a fine vein clearing, accompanied by a marked

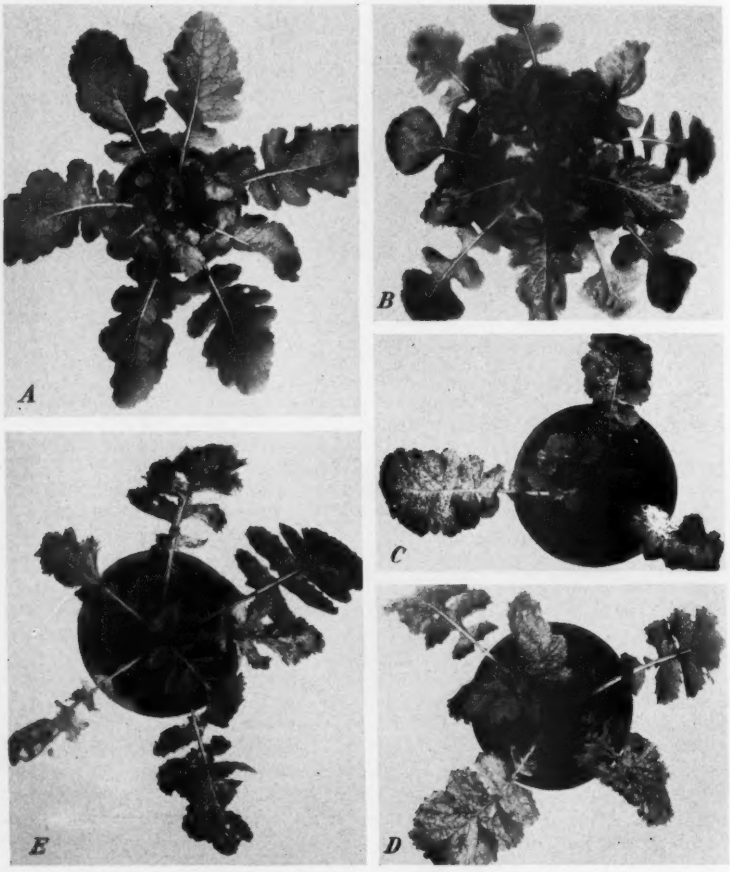

FIGURE 2.—Young turnip plants of the Purple Top White Globe variety inoculated with isolates T1, T6, T8, and T9 and kept in the greenhouse for 20 days at 24° C. *A*, uninoculated control. *B*, Plant inoculated with T1; note coarse mottle on younger leaves with little suppression of growth. *C*, *D*, *E*, Plants inoculated with T6, T8, and T9, respectively; note severe stunting, chlorosis, and leaf malformation with each of these isolates.

crinkling of the youngest leaves. Vein clearing did not persist but was rapidly replaced by a fine interveinal mottle in the case of T6 and T9. With T8, as newer leaves unfolded, they were malformed and severely mottled, becoming chlorotic throughout as they ex-

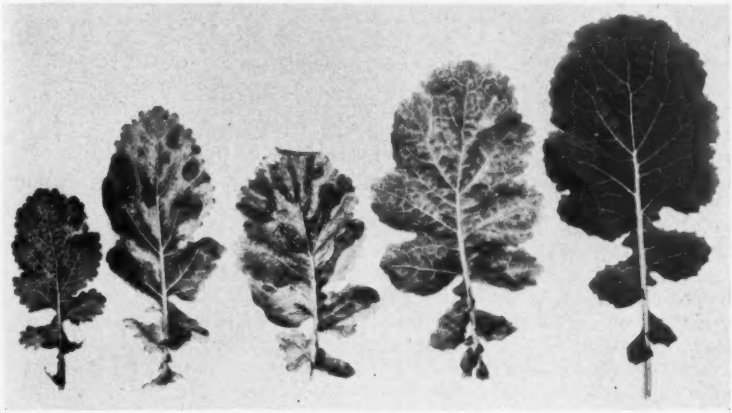

FIGURE 3.—Symptoms on Purple Top White Globe turnip inoculated with isolate T1 and grown at 24° C. for 35 days; leaf from uninoculated plant at right; successively older leaves from inoculated plant, from left to right. Note chlorosis and slight vein clearing in oldest and youngest leaf, with chlorosis and prominent vein banding in the other two leaves.

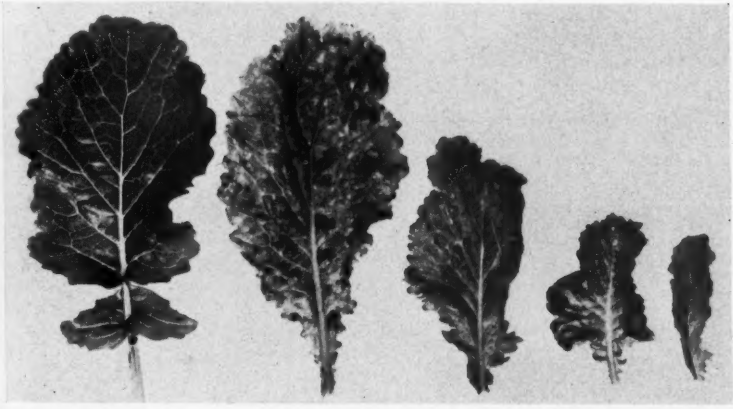

FIGURE 4.—Symptoms on White Milan turnip inoculated with isolate T8 and kept 22 days at 24° C.; leaf from uninoculated plant at left, and successively younger leaves from inoculated plant, from left to right. Note crinkling, malformation, and severe mottling of youngest leaves, with decided chlorosis of older ones.

panded. Stunting was less severe with T9 than with T6 or T8, and the leaves tended to become filiform as they expanded (fig. 4). Necrosis resulting from systemic invasion usually set in on the older leaves in local areas, which rapidly enlarged and coalesced to involve the whole leaf. It was considerably more severe in White Milan than in Purple Top White Globe. T6, T8, and T9 killed many of the former; none of the isolates was lethal to Purple Top White Globe.

RELATION OF TEMPERATURE TO EXPRESSION OF SYMPTOMS

Symptom expression in White Milan and Purple Top White Globe, when infected with the four virus isolates at constant greenhouse temperatures of 16°, 20°, 24°, and 28° C., was studied in three series of tests. Each of the two turnip varieties was most severely affected by each isolate at the highest of these temperatures. At 28° the symptoms did not differ greatly from those at 24°, but they usually appeared earlier and the course of disease development was more rapid. Isolates T6, T8, and T9 were often lethal to the variety White Milan at 24° and 28° but not to Purple Top White Globe. T1 never caused death of plants of either variety. At 16° and 20° there was partial masking of the symptoms produced on each variety by each isolate. At 20° vein clearing, mild mottling, and slight dwarfing occurred in plants infected with T1, T6, and T8; T9 was more severe. At 16° T1, T6, and T8 produced only vein clearing, and mottling appeared but rarely; with T9 vein clearing, mild mottling, and slight stunting were of common occurrence.

PHYSICAL PROPERTIES

The thermal inactivation point, tolerance to dilution, and longevity in vitro were determined for each isolate. The data secured are given in table 1. Since T6, T8, and T9 caused local lesions on tobacco, the local-lesion method was used in all cases with those three viruses and the turnip inoculation test was applied in addition in the thermal inactivation determination. The two methods produced very similar results. T1 was tested only on turnip since tobacco was not infected by this isolate.

TABLE 1—*Properties of 4 turnip viruses as determined by the number of local lesions formed on inoculation to tobacco or the number of systemically infected plants on inoculation to turnip*

Type and degree of treatment	Number of local lesions on tobacco inoculated with virus [1]—			Number of turnip plants infected out of 16 plants inoculated with virus [2]—			
	T6	T8	T9	T6	T8	T9	T1
Undiluted	2,289	1,914	2,004				15
Dilution:							
1–10	303	214	229				15
1–100	82	51	66				15
1–1,000	26	5	18				10
1–2,000		1					
1–5,000	13	0	3				6
1–10,000	1	0	1				3
1–50,000	0	0	0				0
Untreated	3,994	3,706	4,978	14	12	14	14
Heating for 10 minutes (°C.):							
50°	613	265	516	11	7	9	10
52°	256	99	153	9	6	7	11
54°	40	31	41	5	3	4	5
56°	0	1	1	0	1	1	0
58°	0	0	0	0	0	0	0
Inoculated immediately	3,541	2,524	2,134				15
Aging in vitro at 20° C.:							
1 day	1,659	2,323	1,406				
2 days	382	394	212				
3 days	79	264	37				
4 days	33	120	3				10
5 days	6	68	1				8
6 days	4	26	0				6
7 days	0	3	0				6
8 days	0	0	0				3
9 days							1
10 days							0
11 days							0

[1] Average of 3 trials. [2] Average of 2 or 3 trials.

The four isolates were quite similar in reaction to these tests. T8, which will be shown later to conform most closely to cabbage mosaic virus A (*22*), was slightly less tolerant to dilution than T1, T6, or T9. T1 and T6 were inactivated at 56°; T8 and T9 at 58°; T8 was inactivated at 96 hours in vitro at 20°, T6 at 84 hours, T9 at 72 hours, and T1 at 120 hours. It is doubtful that any of these differences are significant, and they could not be depended upon to distinguish between the isolates.

INSECT TRANSMISSION

Each virus isolate was found to be transmissible by the green peach aphid and the cabbage aphid. A high percentage of turnip plants inoculated with either virus isolate by either of the insect species in several trials became infected and produced typical symptoms of the respective virus isolates. Each virus isolate was reisolated by mechanical means from plants infected by aphids. Control plants exposed to nonviruliferous insects remained healthy

HOST RANGE

In table 2 are summarized the results of inoculation of each isolate to a number of cruciferous and noncruciferous plants upon which the reaction of other viruses from cruciferous hosts has been recorded. Since the study on turnip indicated that these were relatively high temperature viruses, these tests were made at 24° and 28° C. It may be seen that T8 infected all botanical varieties of *Brassica oleracea* tested while the other three forms were much more restricted. T1 and T6 infected Italian Green Sprouting broccoli, but did not infect kohlrabi, collard, cabbage, cauliflower, kale, or brussels sprouts. T9 infected kohlrabi and collard as well as Italian Green Sprouting broccoli but did not infect cabbage, cauliflower, kale, or brussels sprouts. All four isolates infected rutabaga, white mustard, black mustard, leaf mustard, rape, and Chinese cabbage, and there was little distinction between the four in symptoms on these hosts except that T1 tended often to produce milder symptoms than any of the other three. Annual stock was infected only by T8 and dames violet only by T8 and T9.

TABLE 2.—*Symptoms produced on young plants in the greenhouse when inoculated mechanically with virus isolates T1, T6, T8, and T9 from turnip*

Host	Symptoms resulting from inoculation with isolate—			
	T1	T6	T8	T9
Cruciferae:				
Brassica oleracea L. var. *capitata* L. (cabbage, var. Jersey Queen).	None.	None.	Local: None. Systemic: Early, slight vein clearing; small, light-green, chlorotic spots, changing to a coarse, irregular mottle; occasional necrosis.	None.
B. oleracea var. *botrytis* L. (cauliflower, var. Early Snowball).	None.	None.	Local: None. Systemic: Same as for cabbage, except no necrosis.	None.
B. oleracea var. *botrytis* L. (broccoli, var. Italian Green Sprouting).	Local: None. Systemic: Very mild chlorotic spots, visible only by transmitted light.	Same as for T8.	Local: None. Systemic: Same as for cabbage, except no necrosis.	Same as for T8.
B. oleracea var. *gongylodes* L. (kohlrabi, var. Early White Vienna).	None.	None.	Local: None. Systemic: Same as for cabbage, except no necrosis.	Local: None. Systemic: Faint, circular, chlorotic spots, visible only by transmitted light; becoming less discernible with age.
B. oleracea var. *viridis* L. (kale, var. Dwarf Scotch Curled).	None.	None.	Local: None. Systemic: Same as for cabbage, except chlorotic spots remain as distinct, light-green, circular areas.	None.
B. oleracea var. *viridis* L. (collard, var. Southern Georgia or Creole).	None.	None.	Local: None. Systemic: Same as for cabbage.	Local: None. Systemic: Same as for kohlrabi.
B. oleracea var. *gemmifera* DC. (Brussels sprouts, var. New Danish).	None.	None.	Local: None. Systemic: Same as cabbage, with necrosis more severe as small, circular, reddish-brown spots on older leaves.	None.

B. campestris L. var. *napobrassica* (L.) DC. (rutabaga, var. American Purple Top).	Local: None. Systemic: Faint, diffuse, chlorotic spots on young leaves, developing into diffuse mottle of faint, chlorotic, and light-green areas.	Same as for T8.	Local: Erratic occurrence of local lesions. Systemic: Some plants become flaccid and leaves necrotic with no mottle; others show pronounced mottle; dwarfing pronounced.	Same as for T8.
B. campestris L. (yellow mustard).	Not tested.	Local: None. Systemic: Same as for T8, except less severe stunting.	Local: None. Systemic: Fine vein clearing on first 3 or 4 leaves; subsequent young leaves show only coarse mottle; severe stunting.	Local: None. Systemic: Same as for T8.
B. hirta Moench (*B. alba* (L.) Rabenh.) white mustard).	Local: None. Systemic: Same as for T8 with less severe stunting.	Local: None. Systemic: Same as for T8 with less severe stunting.	Local: None. Systemic: Same as for yellow mustard.	Local: None. Systemic: Same as for T8.
B. nigra (L.) Koch (black mustard).	Local: None. Systemic: Vein-clearing on successive new leaves; well-defined mottle rare.	Local: None. Systemic: Same as for T8.	Local: None. Systemic: Vein clearing, changing rapidly into severe, fine, yellow, interveinal mottle; progressive yellowing and dying of older to younger leaves, with no other symptoms, or mosaic mottle followed by death or severe stunting.	Local: None. Systemic: same as for T1.
B. juncea (L.) Coss. (leaf mustard, var. Tender Green).	Local: None. Systemic: Vein clearing, continuing on successive leaves; leaf malformation and dwarfing less pronounced than with T8.	Local: None. Systemic: Same as for T8.	Local: None. Systemic: Vein clearing on first 2 or 3 leaves, followed by fine interveinal mottle; subsequent leaves much dwarfed, curled, malformed, chlorotic with dark-green islands.	Local: None. Systemic: Same as for T8.
B. napus L. (rape, var. Dwarf Essex).	Local: None. Systemic: Vein clearing, superseded by very mild chlorosis (associated with absence of bloom) with scattered normal green areas; successive leaves developing coarser mottle.	Local: None. Systemic: Same as for T1.	Local: None. Systemic: Vein clearing, rapidly superseded by interveinal mottle and finally a chlorotic background with scattered, small, green areas, more severe at base and in center of leaf blade; dwarfing.	Local: None. Systemic: Same as for T1.
B. pekinensis (Lour.) Rupr. (Chinese cabbage, var. Chihli).	Local: None. Systemic: No dwarfing or malformation; conspicuous mottle of yellow areas in normal green background or dark-green islands, often puckered, in chlorotic background.	Local: None. Systemic: Infection rare, similar to T8.	Local: None. Systemic: Inconspicuous clearing of veins, rapidly replaced by mottle of diffuse chlorotic spots; subsequent leaves crinkled, savoyed, dwarfed, but not conspicuously mottled; necrosis as small spots or streaks along midrib.	Local: None. Systemic: Same as for T8.

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TABLE 2.—*Symptoms produced on young plants in the greenhouse when inoculated mechanically with virus isolates T1, T6, T8, and T9 from turnips*—Continued

Host	Symptoms resulting from inoculation with isolate—			
	T1	T6	T8	T9
Cruciferae—Continued.				
Hesperis matronalis L. (dames violet).	None.	None.	Local: None. Systemic: Vein clearing in first or second leaf; subsequent leaves chlorotic with dark-green islands or bands along veins; marginal inroll of young leaves; dwarfing.	Local: None. Systemic: Same as for T8.
Matthiola incana (L.) R. Br. var. *annua* (L.) Voss (annual stock, var. Dwarf Ten Weeks).	None.	None.	Local: None. Systemic: Mild interveinal mottle, followed by irregular, necrotic spots and streaks; severe stunting; no flower breaking observed.	None.
Arabis albida Stev. (rockcress).	Not tested.	None.	None.	None.
Cheiranthus allionii Hort. (wallflower).	None.	None.	None.	None.
Non-Cruciferae:				
Nicotiana tabacum L. (tobacco, var. Connecticut Havana 38).	None.	Local: Same as for T8 Systemic: None.	Local: Lesions in 3 to 4 days on inoculated leaves, enlarging rapidly to 3 mm. or more in diameter, usually showing brick-red center with concentric rings and darker band at edge. Systemic: None.	Local: Same as for T8. Systemic: None.
N. glutinosa L.	Local: None. Systemic: Very mild or none; halo chlorotic spotting, followed by occasional necrosis in old leaves; no perceptible dwarfing.	Local: None. Systemic: Same as for T8.	Local: None. Systemic: Severe chlorotic halo spotting, rapidly becoming necrotic.	Local: Circular yellow spots, 2 to 3 mm., not becoming necrotic; irregular in occurrence. Systemic: Same as for T8.
N. tabacum × *N. glutinosa* F_1.	None.	Same as for *N. tabacum*.	Same as for *N. tabacum*.	Same as for *N. tabacum*.
N. rustica L.	None.	None.	Local: None. Systemic: Small, distinct, circular, faintly chlorotic spots, enlarging as leaf expands; little or no malformation or stunting.	Local: None. Systemic: Same as for T8.

N. repanda Lehm.	None.	Local: Same as for T8. Systemic: Same as for T8, except that in later stage necrotic pattern remains that of individual, circular areas.	Local: Small, light brown spots in 3 to 5 days, enlarging to 5 to 8 mm., extending to involve whole leaf. Systemic: Few small, circular, distinct, chlorotic spots, rapidly becoming necrotic; extending to produce irregular, necrotic pattern and streaks along veins.	Local: Same as for T8. Systemic: Same as for T8.
N. sylvestris Speg.	None.	Same as for *N. tabacum*.	Same as for *N. tabacum*.	Same as for *N. tabacum*.
N. multivalvis Lindl.	Not tested.	Local: None. Systemic: Vein-clearing followed by chlorotic spotting.	Local: None. Systemic: Severe etching or necrosis of veins and veinlets.	Local: None. Systemic: Same as for T6.
Petunia hybrida Vilm. (petunia, var. Violet King).	Local: None. Systemic: Indefinite chlorotic banding of larger veins, followed by bright yellow, well-defined spots, few on a leaf; occasional chlorotic bands along large veins; slight dwarfing; no mottle of flower petal.	Local: None. Systemic: Similar to but less severe than T8; flower symptoms not studied.	Local: None. Systemic: Vein clearing, changing into fine, interveinal mottling; dwarfing; conspicuous mottle of flower petals.	Local: None. Systemic: Similar to but less severe than T8; conspicuous mottle of flower petals.
Beta vulgaris L. var. *cicla* L. (Swiss chard, var. Lucullus).	Local: None. Systemic: Same as for T8.	Local: None. Systemic: Same as for T8.	Local: None. Systemic: Erratic necrotic lesions.	Local: None. Systemic: Same as for T8.
B. vulgaris L. (sugar beet, var. Kleinwanzleben).	Same as for Swiss chard.	Same as for Swiss chard.	Same as for Swiss chard.	Same as for Swiss chard.
Spinacia oleracea L. (spinach, var. Bloomsdale).	None.	Local: None. Systemic: Same as for T8.	Local: None. Systemic: Vein clearing, changing to fine, severe, interveinal mottle with severe malformation and dwarfing of leaves.	Local: None. Systemic: Same as T8.
Zinnia elegans Jacq. (Lilliput zinnia, var. Giant Golden).	Local: None. Systemic: Vein clearing, disappearing with age, replaced by chlorotic bands along large veins and by few faint chlorotic spots near base of leaf blade; no stunting.	Local: None. Systemic: Same as for T8.	Local: None. Systemic: Vein clearing, severe, coarse mottle; stunting.	Local: None. Systemic: Same as for T8.

Among noncruciferous plants quite as wide a divergence occurred. T1 did not affect tobacco while T6, T8, and T9 caused only local lesions. All affected *Nicotiana glutinosa* (fig. 5). T9 was the only one

FIGURE 5.—Effect of turnip virus isolates on *Nicotiana glutinosa*. *A*, Circular yellow spots produced by T9 on inoculated leaves after 10 days at 20° C; T1, T6 and T8 did not produce local lesions. *B*, Systemic infection after 20 days at 24° characteristic of T6, T8, and T9; severe chlorotic halos rapidly became necrotic. *C*, Systemic infection by T1 after 22 days at 24°; relatively uncommon chlorotic spots which rarely became necrotic.

to produce local lesions in the form of small yellow spots while systemically it produced symptoms similar to those caused by T8. T6 and T8 caused severe chlorotic halo spots which rapidly became necrotic while T1 uncommonly produced chlorotic spots which rarely

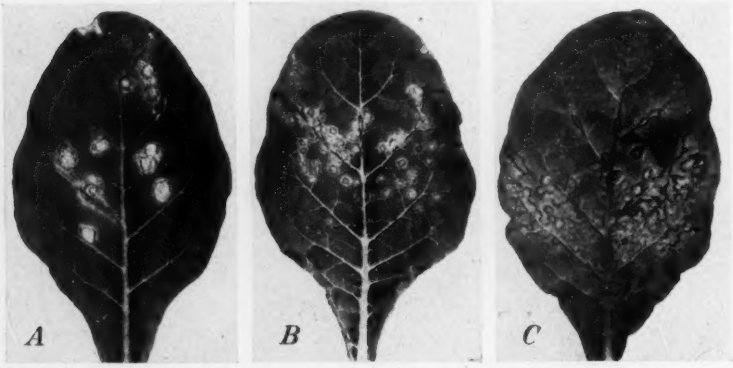

FIGURE 6.—Symptoms on *Nicotiana repanda*. *A*, Local lesions produced on inoculated leaves; these are typical of T6, T8, and T9. *B*, Systemic symptoms of T6; necrotic lesions remain distinct. *C*, Systemic symptoms characteristic of T8 and T9; necrosis of local spots extends to form an irregular pattern and necrosis along veins. T1 causes no infection on *N. repanda*.

became necrotic. T1 and T6 did not affect *N. rustica* and T1 did not affect *N. repanda* or *N. sylvestris*. T6, T8, and T9 produced on *N. repanda* local necrotic lesions which enlarged to 5 to 8 mm. in diameter (fig. 6, *A*). Systemic symptoms appeared as small chlorotic spots which rapidly became necrotic. With T6 these spots remained distinct (fig. 6, *B*), but with T8 and T9 the necrosis extended to produce irregular patterns and streaks along the veins (fig. 6, *C*). A striking difference between isolates was shown in the systemic symptoms produced on *N. multivalvis*. T6 and T9 were similar in that they produced vein clearing followed by chlorotic spotting (fig. 7, *B*); in this

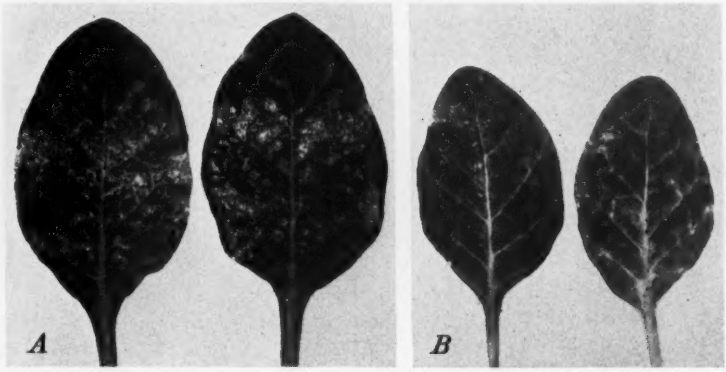

FIGURE 7.—Systemic symptoms on *Nicotiana multivalvis*. *A*, Conspicuous etching pattern caused by isolate T8. *B*, Chlorotic spotting and vein clearing characteristic of T6 and T9.

respect they were similar to cabbage virus A (*22*). T8, which in most hosts produced symptoms similar to cabbage virus A, caused etching and vein necrosis which assumed a striking pattern (fig. 7, *A*) very much like that produced by the cabbage black ring virus on this host (*22*).

All isolates reacted similarly on beet and chard. All affected spinach except T1. All affected petunia and zinnia, but the symptoms produced by T1 differed from those produced by the other three. In zinnia, T1 symptoms consisted of mild, localized chlorosis, while those of T6, T8, and T9 consisted of severe coarse mottle with considerable stunting of affected leaves (fig. 8).

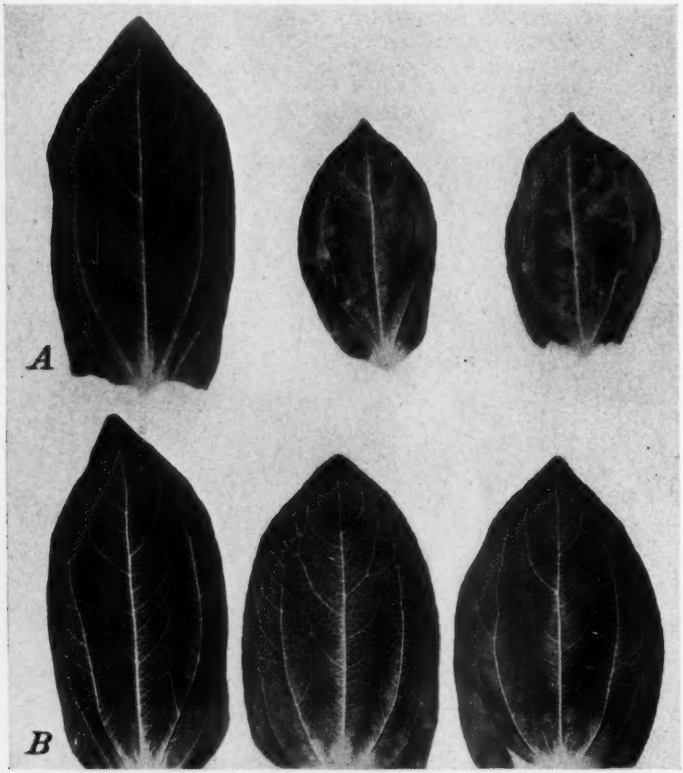

FIGURE 8.—Systemic symptoms on zinnia. *A*, Severe chlorotic mottle characteristic of isolates T6, T8, and T9; leaf from uninoculated plant on left. *B*, Mild localized chlorosis caused by T1; leaf from uninoculated plant on left. Leaves photographed 19 days after inoculation at 24° C.

DISCUSSION

In table 3 are brought together the property reactions and host ranges of the four isolates described in this paper in comparison with others recorded in the literature. When the symptoms produced by T8 are compared with those recently recorded (*22*) for viruses on cabbage, it is evident that T8 is very similar to cabbage virus A. The latter, together with the black ring virus of cabbage, has been classed as a strain of the turnip virus 1 of Hoggan and Johnson (*22*). T8 is undoubtedly another strain of this group. In most respects it produced symptoms similar to cabbage virus A, but on *Nicotiana multivalvis* it produced symptoms almost identical with those produced by the black ring virus (fig. 7).

Insofar as T8, cabbage virus A, the ring necrosis virus, and the black ring virus have been compared, they have been found to have nearly identical host ranges. They differ chiefly in their effects upon

TABLE 3.—*Comparison of properties and host range of isolates T1, T6, T8, and T9 with those of other viruses described from crucifers* [1]

Properties or host range	Turnip virus 1, H and J (8)	Turnip mosaic Tompkins (17)	Turnip mosaic Chamberlain (5)	T1	T6	T8	T9	Cabbage virus A (22)	Cabbage black ring (22)	Cabbage ring necrosis (11)	Radish mosaic (15)	Mild stock mosaic (18)	Severe stock mosaic (18)	Rape mosaic (14)	Cabbage virus B (22)	Cauliflower mosaic (16, 22)	Chinese cabbage mosaic (21)
Properties:																	
Inactivation by dilution	100, 000	4, 000	1, 000	50, 000	50, 000	5, 000	50, 000	4, 000–10, 000	1, 000	600	15, 000	5, 000	4, 000	7, 000	500–1, 500	3, 000	6, 000
Inactivation by ageing (hrs.)	72	72	72	120	84	96	72	-	48	48	384	144	192	144	-	360	96
Inactivation by heat (°C.)	54	63	55–60	55	56	58	58	59–60	60	50	68	60	60	65	74–76	75	75
Host range:																	
Turnip	+	+	+	++	++	++++++++++	++	++++++++++	++++++++++	++++++++++	+	+	+	+	+	++++++++++	+
Rutabaga	+	+	+	++	++	++++++++++	++	++++++++++	++++++++++	++++++++++	+	+	+	+	+	++++++++++	+
Cabbage	-	-	-	0	0	++++++++++	0	++++++++++	++++++++++	++++++++++	+	0	0	-	-	++++++++++	-
Cauliflower	+	++	-	0	0	++++++++++	0	++++++++++	++++++++++	++++++++++	+	0	0	0	-	++++++++++	+
Green sprouting broccoli	0	-	+	0	+	++++++++++	++	++++++++++	++++++++++	++++++++++	+	0	0	-	+	++++++++++	-
Kohlrabi	0	-	0	0	0	++++++++++	0	++++++++++	++++++++++	++++++++++	+	0	0	0	-	++++++++++	-
Kale	0	-	0	0	0	++++++++++	0	++++++++++	++++++++++	++++++++++	+	0	0	-	+	++++++++++	-
Brussels sprouts	0	-	0	0	0	++++++++++	0	++++++++++	++++++++++	++++++++++	+	0	0	-	+	++++++++++	-
Rape	0	-	+	++	++	++++++++++	++	++++++++++	++++++++++	++++++++++	+	0	++	+	+	++++++++++	+
White mustard	0	-	-	++	++	++++++++++	++	++++++++++	++++++++++	++++++++++	+	0	0	-	+	++++++++++	0
Black mustard	-	+	-	++	++	++++++++++	++	++++++++++	++++++++++	++++++++++	+	0	0	-	+	++++++++++	-
Leaf mustard	+	+	-	++	++	++++++++++	++	++++++++++	++++++++++	++++++++++	+	0	0	+	+	++++++++++	0
Radish	0	+	-	+	+	++++++++++	++	++++++++++	++++++++++	++++++++++	+	0	0	+	+	++++++++++	+
Chinese cabbage	+	+	-	+	+	++++++++++	++	++++++++++	++++++++++	++++++++++	+	0	0	+	+	++++++++++	-
Dames violet	-	-	-	-	0	++++++++++	0	++++++++++	++++++++++	++++++++++	-	0	+	-	0	++++++++++	-
Annual stock	+	+	-	0	0	++++++++++	0	++++++++++	++++++++++	++++++++++	0	0	+	-	0	++++++++++	0
Tobacco	L	L	-	0	L	++++++++++	L	++++++++++	++++++++++	++++++++++	L	L	L	L	+	++++++++++	+
Nicotiana glutinosa	L?	L	-	+	L	++++++++++	L	++++++++++	++++++++++	++++++++++	L	L	L	L	0	++++++++++	L
Nicotiana rustica	-	0	-	0	+	++++++++++	+	++++++++++	++++++++++	++++++++++	-	0	0	-	0	++++++++++	L
Nicotiana repanda	-	-	-	0	0	++++++++++	+	++++++++++	++++++++++	++++++++++	-	-	-	-	0	++++++++++	-
Nicotiana sylvestris	-	-	-	0	+	++++++++++	+	++++++++++	++++++++++	++++++++++	-	-	-	-	0	++++++++++	-
Nicotiana multivalvis	-	-	-	-	+	++++++++++	+	++++++++++	++++++++++	++++++++++	-	-	-	-	0	++++++++++	-
Petunia	-	-	-	-	+	++++++++++	+	++++++++++	++++++++++	++++++++++	-	-	-	-	-	++++++++++	-
Chard	-	-	-	+	+	++++++++++	+	+++	++++++++++	++++	-	-	-	-	0	++++++++++	-
Beet	-	-	-	+	+	++++++++++	+	+++	++++++++++	++++	-	-	-	-	0	++++++++++	-
Spinach	-	0	-	0	+	++++++++++	+	+++	++++++++++	++++	+	0	+	-	0	++++++++++	0
Zinnia	-	-	-	+	+	++++++++++	+	-	-	-	-	-	-	-	-	-	-

[1] 0=No symptoms; +=positive systemic symptoms; L=local lesions on inoculated leaves.

the host plants. T1, T6, and T9 closely resemble the above-named in properties, as distinct from the cauliflower mosaic group, but differ from them and from each other in host range and in symptomatology on certain common hosts. In view of information now at hand on other virus groups, such as the cucumber virus group (*24*), it is not surprising to find closely similar crucifer viruses with rather distinct selective host ranges. It is much more logical to consider T1, T6, T8, and T9 as strains of turnip virus 1 than to describe them as distinct viruses.

As stated in another paper (*22*), cabbage virus B, cauliflower mosaic virus, and Chinese cabbage virus, all of which affect turnip, are closely related and are distinct from the turnip virus 1 group. They are more closely restricted to the Cruciferae, they produce more pronounced vein clearing, they have a lower temperature optimum, and a thermal inactivation point some 15° to 20° higher than those of the turnip virus 1 group.

The question may logically be raised as to how certain one may be that isolates T1, T6, T8, and T9 are single-virus strains or what assurance one may have that their apparent differences are not in fact the result of mixtures of one or more strains. It is probable that with the methods employed in this study such a question cannot be answered finally. It should be pointed out, however, that the possibility of mixture with a virus of the cauliflower-mosaic group, which is the usual mixture when one collects a virus extract from cabbage affected with mosaic in the Middle West, has been removed by the thermal inactivation study (table 1). If such a virus were present, there would have been infection after heating much beyond 60° when turnip was used as a test plant. Furthermore, viruses T6, T8, and T9 were each reisolated from local lesions on tobacco and inoculated to turnip with resulting symptoms typical of the original isolate. Stock cultures were then maintained on *Nicotiana glutinosa* to preclude contamination by strains of the cauliflower-mosaic group.

When turnips are found affected with mosaic in nature, any one of the strains so far fully described from crucifers may be suspected as the causal agent. It is also possible that a strain from the turnip virus 1 group and one from the cauliflower-mosaic-virus group may both be present and induce a combined effect. Whether or not wild or cultivated plants in proximity may be infected from turnip will depend upon the strain of virus present. Obviously, in the case of some strains, cabbage and cauliflower, for instance, will not be infected. However, turnip is likely to be readily infected by any of the cruciferous strains that may be present in nearby plants if aphid vectors are available for its transfer.

SUMMARY

In this investigation four virus isolates from turnip (*Brassica rapa* L.), three collected at Madison, Wis., and one at Baton Rouge, La., have been studied in comparison with each other and with previously described viruses from cruciferous plants.

One of the isolates (T8) corresponds very closely in properties and in host range with the strain of turnip virus 1 described as cabbage virus A, and with the black ring and ring necrosis viruses from cabbage. The other three, T1, T6, and T9, are very similar to T8 in properties

and breadth of host range but differ from each other and from T8 in rather important host selectivity. None of the last three infects cabbage, cauliflower, kale, brussels sprouts, or annual stock, though all infect green sprouting broccoli and only T9 infects kohlrabi and dames violet.

T8 and T9 infect all species of Nicotiana tested; T6, all but *N. rustica;* but T1 does not infect *N. tabacum*, *N. rustica*, *N. repanda*, or *N. sylvestris*.

All four infect petunia, chard, beet, and zinnia; all except T1 infect spinach.

It is pointed out that all viruses fully described thus far from crucifers are infectious to turnip. The four isolates described here differ in one point or another from each other and from other viruses described. However, they are considered sufficiently similar to turnip virus 1 to be regarded as strains thereof and are distinct from the cauliflower-mosaic-virus group.

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EFFECTS OF PHOSPHORUS, NITROGEN, AND SOIL MOISTURE ON TOP-ROOT RATIOS OF INBRED AND HYBRID MAIZE[1]

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INTRODUCTION

That changes in environmental factors, such as moisture, nutrients, temperature, oxygen supply, and soil texture, can alter the weight, structure, and direction of growth of plants is common knowledge, yet few investigations have been made to determine how various environmental factors affect the balance of top-to-root growth of plants having different hereditary ratio potentials. The present study was made to determine the reaction of inbred lines and hybrids of maize (*Zea Mays* L.) having genetically different top-root ratios to three controlled environmental factors, namely, phosphorus, nitrogen, and soil moisture.

REVIEW OF LITERATURE

Boonstra (*3*),[2] using seven varieties of peas, found that while top-root ratios generally increase with increases in soil moisture and temperature, the reaction of the various varieties was different. Andrews (*1*) worked with two bush lima beans which differed in root-top ratio. A differential response was found between them with an increase in temperature, while their hybrid usually gave an intermediate response.

Harvey (*4*), studying the response of inbred and hybrid maize to ammonium nitrogen as compared with nitrate nitrogen, found significantly different top-root ratios between strains, but he failed to find any association between top-root ratios and the response of the strains to the different forms of nitrogen.

Smith (*8*), although not working on top-root ratios, noted differential responses among corn inbreds and hybrids on low phosphorus levels. Inbred lines efficient in utilization of low levels of phosphorus had a high ratio of secondary to primary roots, while inefficient lines had a low ratio. The F_1 generation was dominant for both phosphorus efficiency and the high ratio of secondary to primary roots. Increased root absorption due to the inheritance of branched type of root system was given as the explanation for phosphorus efficiency. Lyness (*6*), obtained similar results.

MATERIALS AND METHODS

One white flint and six yellow dent inbreds of maize and several of their F_1 hybrids were used. In previous tests (*7*) four of these

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[2] Italic numbers in parentheses refer to Literature Cited, p. 377.

lines, KR, KR(Osf), Hy, and Ite had exhibited low top-root ratios while the other three, PR, R_4, and WF, had exhibited high ratios.[3]

In each test, plants were grown in the greenhouse from 6 to 8 weeks in completely randomized blocks. Water cultures were used in the phosphorus and nitrogen studies. A modification of the solution used by Smith (*8*) was employed in the phosphorus work, and the buffered solution recommended by Zinzadzé (*10*) for constant pH was used in the nitrogen studies. The solutions were made up as follows:

Modified Smith's solution		Buffered solution (Zinzadzé)	
Salt	*Grams per liter of solution*	*Salt*	*Grams per liter of solution*
KCl	0. 288	KCl	0. 286
$MgSO_4.7H_2O$	. 432	$MgSO_4.7H_2O$	. 500
$Ca(NO_3)_2.4H_2O$	. 469	NH_4NO_3	Varied
$(NH_4)_2SO_4$	. 115	$Ca_3(PO_4)_2$	. 500
KH_2PO_4	Varied		

The method of obtaining the colloidal buffer, $Ca_3(PO_4)_2$, used in Zinzadzé's solution has been described previously (*7*). Iron was added as needed in the form of ferric tartrate. Micronutrients were furnished by impurities in the salts and the tap water used for all cultures. Solutions were changed every 5 to 14 days, depending on the size of the plants. In the phosphorus work, as the solutions were made up, they were adjusted to pH 6.0 by the addition of N/10 H_2SO_4. Quart mason jars were used as containers throughout the water culture experiments.

Plants in the soil moisture studies were grown in glazed gallon jars filled with a compost-soil mixture. Calcium acid phosphate was added to the soil at the rate of 3 gm. per jar. Empty jar weights and dry soil weights were obtained. Field percentage and wilting percentage of the soil were determined according to the methods described by Loomis and Shull (*5*). These were used as criteria in adjusting soil moisture. When the plants were 10 days old a paraffined, 2½-inch clay pot was placed in an inverted position on the surface of each gallon jar and 1 inch of dry washed river sand was spread around the pot on top of the soil to reduce surface evaporation. The plants were watered through the hole in the inverted pot. Jar weight was corrected every 3 or 4 days for green plant weight. The jars were weighed daily in order to adjust for moisture when necessary.

Two plants served as a unit throughout. In harvesting, the roots of soil-grown plants were carefully washed. After harvesting, tops and roots were dried to a constant moisture level and weighed. Top-root ratios were calculated on these weights by dividing the dry weight of the top by the dry weight of the roots. Analyses of variance were carried out on the data obtained according to the method outlined by Snedecor (*9*).

EXPERIMENTAL RESULTS

INFLUENCE OF PHOSPHORUS CONCENTRATION ON INBRED AND HYBRID TOP-ROOT RATIOS

Test 1, conducted from January 25 to March 9, 1940, contained two high- and two low-ratio inbreds and four F_1 hybrids. Phosphorus con-

[3] All lines were obtained through the courtesy of Dr. E. W. Lindstrom.

centrations of 5, 15, and 50 p.p.m. were established. In adjusting KH_2PO_4 to obtain the desired phosphorus supply, KCl was not adjusted to offset the changes in potassium concentration which automatically accompanied changes in the phosphorus level. Mean top-root ratios are presented in table 1 and the analysis of variance is given in table 2.

Lines, treatments, and their interaction were all highly significant. An increase in mean top-root ratios occurred when phosphorus was raised from 5 to 15 p.p.m. but no further increase was found for 50 p.p.m.

TABLE 1.—*Mean top-root ratios of inbreds and hybrids calculated from dry weights; test 1*

Lines	Phosphorus concentration (p. p. m.)			
	5	15	50	Mean
KR	2.00	2.41	2.47	2.29
KR×Hy	1.79	2.02	2.06	1.96
Hy	1.53	2.01	1.96	1.84
PR×Hy	1.92	2.26	2.42	2.20
PR	3.03	3.85	3.89	3.59
PR×R_4	2.37	3.10	2.93	2.80
R_4	2.21	2.80	2.88	2.63
KR×R_4	2.06	2.55	2.31	2.30
Mean	2.11	2.62	2.61	2.45

TABLE 2.—*Analysis of variance of top-root ratios; test 1*

Source of variation	Degrees of freedom	Mean square
Replications	6	0.29
Lines	7	6.55**
Treatments	2	4.79**
Lines×treatments	14	.12**
Error	138	.05
Total	167	

**P less than 0.01.

One cause for the significant interaction of lines by treatments may be seen by comparing the mean differences between hybrids and their high- and low-ratio parents on each phosphorus concentration (table 3). The difference between the high-ratio parents and hybrids when grown on 15 p.p.m. was significantly larger than the corresponding difference on 5 p.p.m., being 0.85 ± 0.37. The same comparison with 50 and 5 p.p.m. gave a difference of 1.28 ± 0.37. The fact that these differences are positive in value indicates that top-root ratios for the high-ratio parents increased faster with increased phosphorus than did those for the hybrids. The same comparison between 50 and 15 p.p.m. was 0.43 ± 0.37. This, though nonsignificant, is positive in value, showing the same trend. Such differences between differences for any two phosphorus concentrations in the "Low-ratio parent minus hybrid" section of table 3 are negative, small, and nonsignificant, indicating that with increased phosphorus supply, hybrids and their low-ratio parents did not respond differently.

The differences between differences (interaction) for the high- and low-ratio inbreds for 15 and 5 p.p.m. of phosphorus were 0.68 ± 0.47

and for 50 and 5 p.p.m., 0.86 ± 0.47. These values are both positive, indicating that the top-root ratios increased with phosphorus concentration more rapidly for the high-than for the low-ratio parents. However, the values are not significant.

In this test each hybrid ratio tended to be nearer to that of its lower ratio parent than to that of its higher ratio parent. However, as a group the hybrid ratios were significantly larger than those of the lower ratio parents (-0.67 ± 0.15). This was also true at each individual phosphorus level (table 3).

TABLE 3.—*Mean differences in top-root ratios between hybrids and their high-ratio parents and between hybrids and their low-ratio parents; test 1*

Hybrid	Phosphorus concentration (p. p. m.)			
	5	15	50	Mean
HIGH-RATIO PARENT MINUS HYBRID				
KR×Hy	+0.21	+0.39	+0.41	+0.34
PR×Hy	+1.11	+1.59	+1.47	+1.39
PR×R_4	+.66	+.75	+.96	+.79
KR×R_4	+.15	+.25	+.57	+.32
Sum	+2.13	+2.98	+3.41	+2.84
Standard error of sum	±.26	±.26	±.26	±.15
LOW-RATIO PARENT MINUS HYBRID				
KR×Hy	−.26	−.01	−.10	−.12
PR×Hy	−.39	−.25	−.46	−.37
PR×R_4	−.15	−.30	−.05	−.17
KR×R_4	−.06	−.14	+.16	−.01
Sum	−.86	−.70	−.45	−.67
Standard error of sum	±.26	±.26	±.26	±.15

Test 2 was planned to check the results of test 1. Because of lack of seed, different inbred lines were used. Phosphorus concentrations were adjusted to 1.67, 5, and 15 p. p. m. Except for these changes the procedure was the same as for test 1. The test was conducted from May 13 to June 20, 1940. Table 4 presents the mean top-root ratios, table 5 the analysis of variance, and table 6 the mean differences between hybrids and their high- and low-ratio parents.

The treatment means show a continuous increase in top-root ratio with increased phosphorus concentration (table 4). As in test 1, the interaction of lines × treatments was highly significant, indicating that all lines did not respond the same to treatment. On breaking down the interaction, several individual comparisons were found to be significant. In the phosphorus concentration range from 1.67 to 5 and from 1.67 to 15 p. p. m. the high-ratio inbred parents of the hybrids increased in top-root ratio more than did the low-ratio parents of the hybrids. (The difference between two differences for each was 1.03 ± 0.52 and 1.92 ± 0.52, respectively.) No significant interactions were found between the hybrids and their low-ratio parents at the various phosphorus concentrations. When the hybrids and their high-ratio parents were compared on the various phosphorus concentrations it was found that values for the latter increased significantly more than the hybrid ratios when phos-

phorus was raised from 1.67 to 5 p.p.m. (1.09±0.42) and from 1.67 to 15 p.p.m. (1.83±0.42). These results confirm previous indications (test 1) that hybrids and their lower ratio parents do not, with increased phosphorus supply, increase in top-root ratio as rapidly as do the higher ratio parents.

In this test, ratios for hybrids were slightly smaller for each phosphorus concentration than were those of their lower ratio parents (table 6). However, none of the mean differences were significant.

TABLE 4.—*Mean top-root ratios of inbreds and hybrids calculated from dry weights; test 2*

Lines	Phosphorus concentration (p. p. m.)			
	1.67	5	15	Mean
KR (Osf)	1.61	2.11	2.38	2.03
KR (Osf)×ITE	1.58	1.70	2.22	1.83
ITE	1.93	1.99	2.28	2.07
ITE×PR	1.65	1.98	2.42	2.02
PR	2.29	3.30	4.02	3.20
PR×WF	2.25	2.60	3.15	2.67
WF	2.80	2.79	3.77	3.12
WF×KR (Osf)	2.08	2.26	2.72	2.35
Mean	2.02	2.34	2.87	2.41

TABLE 5.—*Analysis of variance of top-root ratios; test 2*

Source of variation	Degrees of freedom	Mean square
Replications	7	0.26
Lines	7	6.68**
Treatments	2	11.70**
Lines × treatments	14	.43**
Error	161	.07
Total	191	

**P less than 0.01.

TABLE 6.—*Mean differences in top-root ratios between hybrids and their high-ratio parents and between hybirds and their low-ratio parents; test 2*

HIGH RATIO PARENT MINUS HYBRID

Hybrid	Phosphorus concentration (p.p.m.)			Mean
	1.67	5	15	
KR (Osf)×ITE	+0.35	+0.29	+0.06	+0.23
ITE×PR	+.64	+1.32	+1.60	+1.19
PR×WF	+.04	+.70	+.87	+.54
WF×KR (Osf)	+.72	+.53	+1.05	+.77
Sum	+1.75	+2.84	+3.58	+2.73
Standard error of sum	±.30	±.30	±.30	±.17

LOW-RATIO PARENT MINUS HYBRID

KR (Osf)×ITE	+.03	+.41	+.16	+.20
ITE×PR	+.28	+.01	−.14	+.05
PR×WF	+.55	+.19	+.62	+.44
WF×KR (Osf)	−.47	−.15	−.34	−.32
Sum	+.39	+.46	+.30	+.37
Standard error of sum	±.30	±.30	±.30	±.17

INFLUENCE OF NITROGEN CONCENTRATION ON INBRED AND HYBRID TOP-ROOT RATIOS

Two tests were conducted to determine whether inbred and hybrid top-root ratios would respond to various concentrations of nitrogen in the same way that they had to phosphorus.

Test 3 was made with four inbreds, consisting of two low-ratio and two high-ratio lines, and four single crosses. Nitrogen levels of 5, 15, 45, and 135 p.p.m. were established. The plants were grown from December 26, 1940, to February 12, 1941. The mean top-root ratios are given in table 7, the analysis of variance in table 8, and the mean differences between hybrids and their high- and low ratio parents in table 9.

The treatment means increased steadily with nitrogen concentration. By subdividing the sum of squares for treatment into its linear, quadratic, and cubic parts, the mean squares obtained are 19.47, 0.87, and 0.25, respectively, showing that the bulk of the increase was linear.

In spite of the significant interaction of lines × treatments, no significant interaction between high- and low-ratio inbreds, between hybrids and their low-ratio parents, or between hybrids and their high-ratio parents was found. Apparently nitrogen caused no differential response among the three comparisons and the significance of the interaction variance was due to other causes.

In this test, hybrid ratios did not differ from those of the low-ratio parents, either for the test as a whole (-0.11 ± 0.12) or for any individual nitrogen level. They were significantly lower in all cases than those of the high-ratio parents.

TABLE 7.—*Mean top-root ratios of inbreds and hybrids calculated from dry weights; test 3*

Lines	Nitrogen concentration (p.p.m.)				Mean
	5	15	45	135	
KR (Osf)	1.20	1.38	1.60	2.25	1.60
Kr (Osf)×R_4	1.65	1.78	2.01	2.59	2.01
R_4	2.44	2.77	2.78	[1] 2.97	2.74
R_4×PR	1.98	2.27	2.59	3.00	2.46
PR	2.31	2.60	2.95	3.61	2.87
PR×Hy	1.48	1.73	1.87	2.33	1.85
Hy	1.63	1.68	1.77	2.16	1.81
Hy×KR (Osf)	1.20	1.40	1.55	2.09	1.56
Mean	1.74	1.95	2.14	2.62	2.11

[1] 1 plant unit lost by disease. Datum supplied by missing plot technique.

TABLE 8.—*Analysis of variance of top-root ratios; test 3*

Source of variation	Degrees of freedom	Mean square
Replications	5	0.16
Lines	7	6.24**
Treatments	3	6.87**
Lines×treatments	21	.10**
Error	[1] 154	.03
Total	190	

**P less than 0.01.
[1] 1 degree of freedom subtracted for datum supplied by missing plot technique.

TABLE 9.—*Mean differences in top-root ratios between hybrids and their high-ratio parents and between hybrids and their low-ratio parents; test 3*

HIGH RATIO PARENT MINUS HYBRID

Hybrid	Nitrogen concentration (p.p.m.)				Mean
	5	15	45	135	
KR (Osf) × R_4	+0.79	+0.99	+0.77	+0.38	+0.73
R_4 × PR	+.33	+.33	+.36	+.61	+.41
PR × Hy	+.83	+.87	+1.08	+1.28	+1.01
Hy × KR (Osf)	+.43	+.28	+.22	+.07	+.25
Sum	+2.38	+2.47	+2.43	+2.34	+2.40
Standard error of sum	±.23	±.23	±.23	±.23	±.12

LOW RATIO PARENT MINUS HYBRID

KR (Osf) × R_4	−.45	−.40	−.41	−.34	−.40
R_4 × PR	+.46	+.50	+.19	−.03	+.28
PR × Hy	+.15	−.05	−.10	−.17	−.04
Hy × KR (Osf)	.00	−.02	+.05	+.16	+.05
Sum	+.16	+.03	−.27	−.38	−.11
Standard error of sum	±.23	±.23	±.23	±.23	±.12

In test 4 the same lines were grown on the nitrogen levels of 4, 16, 64, and 256 p.p.m. As a precaution against toxicity from the ammonium ion of the ammonium nitrate, 0.5 p.p.m. of manganous chloride was added to the nutrient solution (*2*). The experiment was conducted from March 22 to April 30, 1941. Tables 10 and 11 present the data and analysis, respectively, and table 12, the mean differences.

The results of this test tend to substantiate the findings of the previous nitrogen experiments. Although the interaction of lines × treatments was again significant, no consistent interaction existed between the high- and low-ratio parents of the hybrids or between either group of parents and the hybrids.

Again hybrid ratios did not differ significantly from those of the low-ratio parents for any level of nitrogen except 64 p.p.m. No reason is known why this concentration should have caused a significant difference.

INFLUENCE OF SOIL MOISTURE ON INBRED AND HYBRID TOP-ROOT RATIOS

To determine how inbred and hybrid ratios respond to different concentrations of soil moisture, two tests were conducted in which the percentage of water in the soil was controlled.

In test 5, three inbreds and three hybrids were grown from October 15 to December 4, 1940, at moisture levels of 8–17, 11–17, and 24 percent. The field percentage of the soil used was 16.2, so it was necessary, for the first two groups, to successively add enough water to bring the soil moisture to 17 percent and then to allow the plants to reduce it to the lower moisture percentage before more was added. (The wilting percentage was 8.06). For the third level, jars were weighed daily and kept at 24 percent moisture. The results of the test are presented in tables 13, 14, and 15.

TABLE 10.—*Mean top-root ratios of inbreds and hybrids calculated from dry weights; test 4*

Lines	Nitrogen concentration (p.p.m.)				Mean
	4	16	64	256	
KR (Osf)	0.86	1.07	1.34	1.89	1.29
KR (Osf) × R_4	1.33	1.50	1.99	2.04	1.71
R_4	2.10	2.76	2.42	2.54	2.45
R_4 × PR	1.70	2.14	2.14	2.63	2.15
PR	2.04	2.39	2.68	3.25	2.59
PR × Hy	1.23	1.43	1.57	2.04	1.57
Hy	1.25	1.43	1.36	[1] 1.73	1.44
Hy × KR (Osf)	.92	1.08	1.30	1.55	1.21
Mean	1.43	1.72	1.85	2.21	1.80

[1] 1 plant unit lost by disease. Datum supplied by missing plot technique.

TABLE 11.—*Analysis of variance of top-root ratios; test 4*

Source of variation	Degrees of freedom	Mean square
Replications	5	0.08
Lines	7	6.79**
Treatments	3	4.99**
Lines×treatments	21	.18**
Error	[1] 154	.02
Total	190	

[1] 1 degree of freedom subtracted for datum supplied by missing plot technique.
**P less than 0.01.

TABLE 12.—*Mean differences in top-root ratios between hybrids and their high-ratio parents and between hybrids and their low-ratio parents, test 4*

HIGH-RATIO PARENT MINUS HYBRID

Hybrid	Nitrogen concentration (p.p.m.)				Mean
	4	16	64	256	
KR (Osf)×R_4	+0.77	+1.26	+0.43	+0.50	+0.74
R_4×PR	+.34	+.25	+.54	+.62	+.44
PR×Hy	+.81	+.96	+1.11	+1.21	+1.02
Hy×KR (Osf)	+.33	+.35	+.06	+.18	+.23
Sum	+2.25	+2.82	+2.14	+2.51	+2.43
Standard error of sum	±.16	±.16	±.16	±.16	±.08

LOW-RATIO PARENT MINUS HYBRID

KR (Osf)×R_4	−.47	−.43	−.65	−.15	−.43
R_4×PR	+.40	+.62	+.28	−.09	+.30
PR×Hy	+.02	.00	−.21	−.31	−.12
Hy×KR (Osf)	−.06	−.01	+.04	+.34	+.08
Sum	−.11	+.18	−.54	−.21	−.17
Standard error of sum	±.16	±.16	±.16	±.16	±.08

TABLE 13.—*Mean top-root ratios of inbreds and hybrids calculated from dry weights; test 5*

Lines	Percent soil moisture			Mean
	8–17	11–17	24	
WF	2.91	3.47	5.60	3.99
WF×Hy	3.16	3.12	4.29	3.52
Hy	3.24	3.40	4.36	3.67
PR×Hy	3.47	3.53	4.21	3.74
PR	5.83	5.42	6.97	6.07
PR×WF	3.42	3.64	5.49	4.18
Mean	3.67	3.76	5.15	4.20

TABLE 14.—*Analysis of variance of top-root ratios; test 5*

Source of variation	Degrees of freedom	Mean square
Replications	5	0.17
Lines	5	16.19**
Treatments	2	24.83**
Lines×treatments	10	.95**
Error	85	.17
Total	107	

**P less than 0.01.

TABLE 15.—*Mean differences in top-root ratios between hybrids and their high-ratio parents and between hybrids and their low-ratio parents, test 5*

HIGH-RATIO PARENT MINUS HYBRID

Hybrid	Percent soil moisture			Mean
	8–17	11–17	24	
WF×Hy	−0.25	+0.35	+1.31	+0.47
PR×Hy	+2.36	+1.89	+2.76	+2.34
PR×WF	+2.41	+1.78	+1.48	+1.89
Sum	+4.52	+4.02	+5.55	+4.70
Standard error of sum	±.47	±.47	±.47	±.27

LOW-RATIO PARENT MINUS HYBRID

WF×Hy	+.08	+.28	+.07	+.14
PR×Hy	−.23	−.13	+.15	−.07
PR×WF	−.51	−.17	+.11	−.19
Sum	−.66	−.02	+.33	−.12
Standard error of sum	±.47	±.47	±.47	±.27

The mean top-root ratio for the lowest moisture level was 3.67, and for the intermediate level, 3.76. When these two means were compared they were found not to differ significantly, indicating that the effects of the treatment occurred between these levels and the high moisture level.

The interaction of lines×treatments was highly significant. No difference was found in the interaction of the high- and low-ratio hybrid parents with moisture levels. Nor was any interaction found

between the hybrids and their low-ratio parents. Therefore the significance of the interaction variance must have been caused by different reactions to treatment within the groups of inbreds and hybrids.

Mean differences between hybrid ratios and their low-ratio parents were nonsignificant, whether calculated for the experiment as a whole or for each individual moisture level.

Test 6 was similar to test 5 except that another moisture level was added and it was conducted at a different season of the year. The saturation percentage, not determined in the first test, and the field and wilting percentages of the soil used, were, respectively, 34.7, 14.5, and 7.0 percent. This test was conducted from April 23 to May 29, 1941. Tables 16, 17, and 18 present the results of the test.

TABLE 16.—*Mean top-root ratios of inbreds and hybrids calculated from dry weights; test 6*

Lines	Percent soil moisture				Mean
	7.5–15.5	11.0–15.5	17.5	20.8	
WF	1.99	2.57	2.68	3.41	2.66
WF×Hy	2.44	2.52	2.17	2.71	2.46
Hy	2.15	2.85	2.80	3.09	2.72
PR×Hy	2.41	2.71	2.71	2.75	2.64
PR	3.42	3.68	4.15	4.98	4.06
PR×WF	2.40	2.65	2.80	3.46	2.83
Mean	2.47	2.83	2.88	3.40	2.90

TABLE 17.—*Analysis of variance of top-root ratios; test 6*

Source of variation	Degrees of freedom	Mean square
Replications	4	0.46
Lines	5	6.77**
Treatments	3	4.42**
Lines×treatments	15	.37**
Error	92	.06
Total	119	

**P less than 0.01.

TABLE 18.—*Mean differences in top-root ratios between hybrids and their high-ratio parents and between hybrids and their low-ratio parents; test 6*

HIGH-RATIO PARENT MINUS HYBRID

Hybrid	Percent soil moisture				Mean
	7.5–15.5	11.0–15.5	17.5	20.8	
WF×Hy	−0.29	+0.33	+0.63	+0.38	+0.26
PR×Hy	+1.01	+.97	+1.44	+2.23	+1.41
PR×WF	+1.02	+1.03	+1.35	+1.52	+1.23
Sum	+1.74	+2.33	+3.42	+4.13	+2.90
Standard error of sum	±.32	±.32	±.32	±.32	±.16

LOW-RATIO PARENT MINUS HYBRID

WF×Hy	−.45	+.05	+.51	+.70	+.20
PR×Hy	−.26	+.14	+.09	+.34	+.08
PR×WF	−.41	−.08	−.12	−.05	−.17
Sum	−1.12	+.11	+.48	+.99	+.11
Standard error of sum	±.32	±.32	±.32	±.32	±.16

The results were similar to those of test 5. While interaction of lines × treatments was highly significant, no interaction was found between moisture levels and the high- and low-ratio lines used as hybrid parents. Hybrids differed from their low-ratio parents in their reaction to soil moisture only when comparisons were made involving the lowest moisture level. The mean difference between the low-ratio parents and hybrids at the low moisture level was -1.65 ± 0.37 less than the average mean difference between low inbreds and hybrids for the other three moisture levels. The same comparison of differences for the hybrids and their high-ratio parents was -1.55 ± 0.37. These values are highly significant. Why the reaction of hybrids and inbreds at this soil-moisture level should differ from that at the other levels is not apparent.

No differences were found between the total mean top-root ratios of the hybrids and those of the low-ratio parents. When compared on the basis of individual moisture percentages they differed only on the lowest level, the hybrids having a larger ratio than the inbreds.

DISCUSSION

Top-root ratios increased for both hybrids and their parental inbreds as phosphorous was raised from limiting to optimum concentrations. No significant differences in rate of increase between the hybrids and their lower ratio parents occurred. When rate of hybrid increase was compared with that of the higher ratio parents, it was found that, as the phosphorus level was raised, ratios for these inbreds increased faster than those for the hybrids; in other words, as phosphorus became limiting, top-root ratios decreased more rapidly for the high-ratio parents than for the hybrids or their low-ratio parents.

As previously stated, Smith (*8*) found a high ratio of secondary to primary roots in phosphorus-efficient inbred lines of corn and a low ratio with phosphorus-inefficient lines. He advanced the theory that phosphorus efficiency lies in a large absorbing surface, a factor of importance to the plant since phosphorus moves very little in the soil solution. Hence, to grow well on soils low in phosphorus the plant needs a ramifying root system. In the nutrient solution used, in which phosphorus becomes quickly precipitated out of solution, the plant with the larger absorptive area would have the advantage in absorbing phosphorus before it became unavailable.

When the quantity of any substance absorbed by the roots, i. e., any nutrient or soil moisture, is insufficient for optimum growth, the largest part of that absorbed is used (along with organic foods translocated from the tops) in root growth. Top growth is thereby relatively more limited than root growth, resulting in low top-root ratios. Inversely, when the amount of the substance absorbed by the roots is greater, relatively larger quantities are translocated to the tops, where it is utilized in top growth. Under these conditions most of the organic foods produced in the tops are utilized in their growth and very little is translocated to the roots, which results in larger top-root ratios.

Therefore, if a correlation between root weight and root-absorbing surface exists, plants with the lowest top-root ratio (i. e., with the most roots and therefore with the greatest absorbing surface per unit of

top weight) will absorb the most phosphorus per weight of top, expecially if phosphorus is low, and the result will be less phosphorus deficiency and less reduction in top growth. This in turn would lead to a smaller reduction in top-root ratios, or, inversely, to smaller increases with added phosphorus, when the initial supply is limited. Hybrids with top-root ratios similar to those of their low-ratio parents would respond like their parents. The results of both tests 1 and 2 confirm this interpretation.

Hybrid and inbred ratios responded similarly to various nitrogen levels, the rate of ratio increase as nitrogen was raised from a low to a high concentration being similar for hybrids, for their low-ratio parents, and for their high-ratio parents. This would be expected since nitrogen is not precipitated out of solution in water cultures and should be equally available to all plants regardless of the type of their root system.

Hybrid and inbred top-root ratios increased similarly as soil moisture was raised from low to high levels. Although the water supply may be reduced below that necessary for optimum growth, causing lowered top-root ratios (tops suffering the most, being farthest from the supply), roots will continue to grow throughout the soil, obtaining moisture until it is reduced to a point where it is comparatively unavailable (approximately the wilting percentage). If soil moisture is reduced to the same level for all plants before being replenished, there is no reason to assume that the top-root ratio decrease would be proportionally more for a plant with a large ratio (i. e., few roots) than for a plant with a small ratio.

The results of these tests add weight to information already reported (7) that hybrid top-root ratios either did not differ or differed only slightly from those of their lower ratio parents. This was true regardless of whether the hybrid consisted of two high-, two low-, or a high- and a low-ratio inbred.

SUMMARY

Studies were made to determine the top-root ratios of maize hybrids, their low-ratio parents, and their high-ratio parents at different controlled concentrations of phosphorus, nitrogen, and soil moisture. In each experiment three or four concentrations were used, ranging from those definitely limiting, to those ample for good plant growth.

Top-root ratios increased with phosphorus concentration, but did not increase as rapidly (measured arithmetically) for hybrids or their low-ratio parents as for their high-ratio parents. No difference in rate of ratio increase was found between hybrids and their low-ratio parents.

The explanation is advanced that hybrids and their low-ratio parents, having more roots per unit of top weight than the high-ratio parents, absorb proportionally more phosphorus before it is precipitated out of the nutrient solution, and therefore suffer less phosphorus starvation when the phosphorus supply is low. This would result in less reduction in top-root ratio for the hybrids and low-ratio lines when grown on limited supplies of phosphorus and consequently a relatively smaller increase with additional phosphorus.

Top-root ratios increased with an increase in nitrogen concentration but no differential rate of arithmetic increase was found between

hybrids and either their high- or low-ratio parents. The results when soil moisture was increased were similar to those with nitrogen.

In all tests F_1 hybrid ratios either did not differ or differed only slightly from those of their lower ratio parents.

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